

Presence of Middle East respiratory syndrome coronavirus antibodies in Saudi Arabia: a nationwide, cross-sectional, serological study



Marcel A Müller, Benjamin Meyer, Victor M Corman, Malak Al-Masri, Abdulhafeez Turkestani, Daniel Ritz, Andrea Sieberg, Souhaib Aldabbagh, Berend-J Bosch, Erik Lattwein, Raafat F Alhakeem, Abdullah M Assiri, Ali M Albarak, Ali M Al-Shangiti, Jaffar A Al-Tawfiq, Paul Wikramaratna, Abdullah A Alrabeeah, Christian Drosten*, Ziad A Memish*

Summary

Background Scientific evidence suggests that dromedary camels are the intermediary host for the Middle East respiratory syndrome coronavirus (MERS-CoV). However, the actual number of infections in people who have had contact with camels is unknown and most index patients cannot recall any such contact. We aimed to do a nationwide serosurvey in Saudi Arabia to establish the prevalence of MERS-CoV antibodies, both in the general population and in populations of individuals who have maximum exposure to camels.

Methods In the cross-sectional serosurvey, we tested human serum samples obtained from healthy individuals older than 15 years who attended primary health-care centres or participated in a national burden-of-disease study in all 13 provinces of Saudi Arabia. Additionally, we tested serum samples from shepherds and abattoir workers with occupational exposure to camels. Samples were screened by recombinant ELISA and MERS-CoV seropositivity was confirmed by recombinant immunofluorescence and plaque reduction neutralisation tests. We used two-tailed Mann Whitney *U* exact tests, χ^2 , and Fisher's exact tests to analyse the data.

Findings Between Dec 1, 2012, and Dec 1, 2013, we obtained individual serum samples from 10 009 individuals. Anti-MERS-CoV antibodies were confirmed in 15 (0·15%; 95% CI 0·09–0·24) of 10 009 people in six of the 13 provinces. The mean age of seropositive individuals was significantly younger than that of patients with reported, laboratory-confirmed, primary Middle Eastern respiratory syndrome (43·5 years [SD 17·3] vs 53·8 years [17·5]; $p=0\cdot008$). Men had a higher antibody prevalence than did women (11 [0·25%] of 4341 vs two [0·05%] of 4378; $p=0\cdot028$) and antibody prevalence was significantly higher in central versus coastal provinces (14 [0·26%] of 5479 vs one [0·02%] of 4529; $p=0\cdot003$). Compared with the general population, seroprevalence of MERS-CoV antibodies was significantly increased by 15 times in shepherds (two [2·3%] of 87, $p=0\cdot0004$) and by 23 times in slaughterhouse workers (five [3·6%] of 140; $p<0\cdot0001$).

Interpretation Seroprevalence of MERS-CoV antibodies was significantly higher in camel-exposed individuals than in the general population. By simple multiplication, a projected 44 951 (95% CI 26 971–71 922) individuals older than 15 years might be seropositive for MERS-CoV in Saudi Arabia. These individuals might be the source of infection for patients with confirmed MERS who had no previous exposure to camels.

Funding European Union, German Centre for Infection Research, Federal Ministry of Education and Research, German Research Council, and Ministry of Health of Saudi Arabia.

Introduction

As of March 8, 2015, Middle East respiratory syndrome coronavirus (MERS-CoV) has caused at least 1082 mostly severe cases of respiratory infection, 439 of these fatal, since its discovery in 2012.¹ Apart from its geographical focus in countries in and around the Arabian Peninsula and laboratory evidence of widespread infection of dromedary camels, little is known about the actual epidemiology of the disease in human beings.^{1–5} Few primary infections were acquired through direct camel exposure, but the relevance of this infection pattern is unclear in the absence of systematic determinations of the proportion of infections in individuals who have had contact with camels.^{5–7} Moreover, most reported patients with primary MERS-CoV infection have no history of

camel exposure, suggesting the existence of other, as-yet-unknown sources of infection. Camel milk as a food-borne source seems unlikely.⁸

For secondary infections acquired through human-to-human contact, mathematical projections predicted that transmission chains in the population cannot be sustained.^{9–11} The apparent transmission rate in household settings is low, with fewer than 50% of index patients transmitting the infection to contacts who subsequently had no pronounced clinical symptoms.¹² However, a highly fatal outbreak of MERS-CoV infection centred in Jeddah, Saudi Arabia, in March–April, 2014, was apparently caused by human-to-human transmission in several nosocomial settings, without any evidence that the causative virus differed from other MERS-CoV strains

Lancet Infect Dis 2015;
15: 559–64

Published Online
April 9, 2015
[http://dx.doi.org/10.1016/S1473-3099\(15\)70090-3](http://dx.doi.org/10.1016/S1473-3099(15)70090-3)

See Comment page 495

*Contributed equally
Institute of Virology,
University of Bonn Medical
Centre, Bonn, Germany
(M A Müller PhD, B Meyer MSc,
V M Corman MD, D Ritz MSc,
A Sieberg BSc, S Aldabbagh DVM,
Prof C Drosten MD); German
Centre for Infection Research,
Partner Site Bonn-Cologne,
Bonn, Germany (Prof C Drosten,
V M Corman); Saudi Center for
Disease Control
(A M Albarak MD), and
National Health Laboratory,
(A M Al-Shangiti PhD), King
Abdulaziz Medical City &
Advisor Royal Court, Riyadh,
Saudi Arabia (A Alrabeeah MD,
M Al-Masri BSc, A Turkestani MD,
R F Alhakeem MD, A M Assiri MD,
Prof Z A Memish MD); Makkah
Regional Health Affairs,
Ministry of Health, Makkah,
Saudi Arabia (A Turkestani MD);
Department of Infectious
Diseases and Immunology,
Faculty of Veterinary Medicine,
Utrecht University, Utrecht,
Netherlands (B-J Bosch PhD);
Euroimmun AG, Lübeck,
Germany (E Lattwein PhD);
Johns Hopkins Aramco
Healthcare, Dhahran, Saudi
Arabia (J A Al-Tawfiq MD);
Indiana University School of
Medicine, Indianapolis, IN, USA
(J A Al-Tawfiq); Institute of
Evolutionary Biology,
University of Edinburgh,
Centre for Infection, Immunity
and Evolution, Edinburgh, UK
(P Wikramaratna PhD); and
Alfaisal University, Riyadh,
Saudi Arabia (Prof Z A Memish)

Correspondence to:
Prof Ziad A Memish, Ministry of Health, Preventive Medicine, PO Box 54146, Riyadh 11514, Saudi Arabia
zmemish@yahoo.com

in people, in terms of replication in cell culture, immune escape, or excretion level.¹³ Undiscovered MERS-CoV infections might thus exist in the human population.

Serology is key to the understanding of infection statistics at a population level. However, only a few serological studies of MERS-CoV have been done.^{14,15} Although none of those preliminary studies showed any evidence of previous infection with MERS-CoV in the population of Saudi Arabia, the examined cohorts have been small and methods applied in those studies have not been validated for sensitivity and specificity. We previously presented the first validated recombinant ELISA (rELISA) for MERS-CoV and combined this test with recombinant immunofluorescence assay (rIFA) and a plaque reduction virus neutralisation test (PRNT) into a staged strategy for MERS-CoV serology that was shown to identify even subclinical infections on detection of specific antibodies.¹² Here, we apply the established testing algorithm on a nationwide serum collection. Additional serum samples were obtained from individuals occupationally exposed to dromedary camels.

Methods

Sample collection

For the cross-sectional serosurvey we used existing sample collections organised by the Ministry of Health of Saudi Arabia. Individual serum samples were obtained from healthy people older than 15 years, selected to represent all ages (excluding children and adolescents <15 years) in the Saudi Arabian population. Samples were obtained with written informed consent given to the Ministry of Health from participants in households randomly selected in a population-based, cross-sectional sampling frame maintained and updated by the National Census Bureau.¹⁶ Participants lived and gave samples in all 13 provinces of Saudi Arabia. Sampling was done through primary health-care centres and hospitals under the supervision of the Ministry of Health. We obtained two collections of serum samples from local primary health-care clinics for individuals selected to participate in a survey of the national burden of disease,^{16,17} and a second collection of samples from individuals selected for a national study of the seroprevalence of herpes simplex virus.

Additionally, we obtained serum samples from camel shepherds who participated in an ad hoc MERS-CoV surveillance study implemented by the Ministry of Health and expatriate slaughterhouse workers in Makkah. The shepherds gave verbal consent and the slaughterhouse workers gave direct written consent in the frame of the ad hoc surveillance study.

Procedures

All serum samples were anonymised before the study and were tested for MERS-CoV antibodies without further institutional review, because the testing of all samples was done as part of a public health intervention by the Saudi Ministry of Health. We applied an anti-MERS-CoV ELISA

IgG (EUROIMMUN AG, Lübeck, Germany) with minor modifications. The rELISA was based on the soluble MERS-CoV spike protein subdomain S1.¹⁸ This highly sensitive assay has been validated for use with human serum samples.¹² Samples were applied at a 1/100 dilution. The cutoff optical density ratio was set to 0·3, which is three times the ratio of the mean value of all tested human serum samples without known exposure to camels.

We used rIFA to substantiate rELISA results because rIFA has been shown to be more specific than rELISA.¹² rIFA was done with the full MERS-CoV spike open-reading frame expressed in VeroB4 cells.^{12,19} For IgG detection, serum samples were tested at a 1/40 dilution. For IgM detection, samples were analysed at 1/20 and 1/100 dilutions.

We did plaque reduction neutralisation tests (PRNT) as previously described.²⁰ The first applied dilution of a two-fold dilution series was 1/10. In accordance with our previous study,¹² we defined a 1/20 dilution as the lowest possible diagnostically significant titre.²⁰ We recorded serum dilutions conferring 50% and 90% plaque reduction as PRNT₅₀ and PRNT₉₀ titres, respectively.

We defined stage 1 seropositivity as reactivity in both rELISA (dilution 1/100) and rIFA (dilution 1/40). We defined stage 2 seropositivity as stage 1 seropositivity plus reactivity in PRNT with a reciprocal titre of at least 20 on PRNT₅₀. We regarded stage 2 seropositive results as serological confirmation of past MERS-CoV infection.

To compare the age distribution of patients with serologically confirmed cases with that of patients with clinically apparent cases, we examined data from international case-notification statistics²¹ and index-case data from a study of household contact clusters.¹²

Statistical analysis

We did a two-tailed Mann Whitney *U* exact test with SPSS software (version 21). We did χ^2 tests and Fisher's exact tests, and calculated CIs with OpenEpi (Open Source Epidemiologic Statistics for Public Health). We applied χ^2 tests in the seroprevalence calculations of the general population versus camel-exposed individuals and for comparisons of agegroups. We used Fisher's exact tests for calculations of geographical and gender comparisons. We applied the non-parametric Mann Whitney *U* test to compare the mean ages of patients with MERS in different studies.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between April 1, and June 30, 2013, we obtained 5765 serum samples from local primary health-care

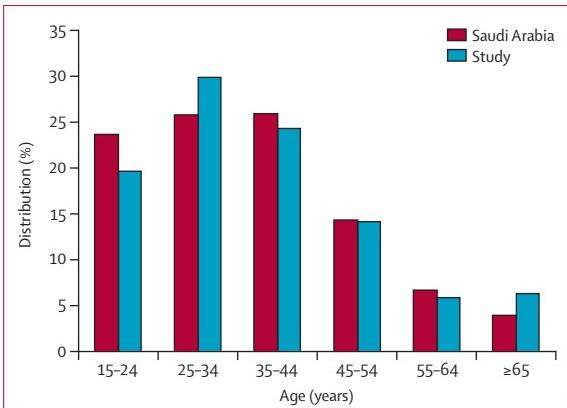


Figure 1: Age distribution of the study sample compared with the total population

Census for the population of Saudi Arabia in 2013 was retrieved from the Saudi Arabia Central Department of Statistics and Information (detailed numbers in the appendix).

clinics for individuals selected to participate in a survey of the national burden of disease,^{16,17} and a further 4244 samples between Dec 1, 2012, and Dec 1, 2013 from primary health-care centres. Data for patient demographic characteristics (appendix) were available for 8719 (87%) of 10 009 stored serum samples.

Additionally, we obtained serum samples from 87 camel shepherds who participated in a MERS-CoV surveillance study from Feb 1, to May 31, 2014, and 140 expatriate slaughterhouse workers in Makkah during August, 2013. The camel shepherds were aged 21–84 years (mean 37·9 years, SD 13·02) and resided in six different provinces (Al-Jouf, Asir, Eastern, Madinah, Makkah, and Riyadh). The slaughterhouse workers were aged 15–62 years (mean 37·13, SD 8·64).

The mean sample size per province was 770 (range 321 to 2343 [SD 585]), representing between 19·7 and 97·1 samples per 100 000 individuals per province (mean 52·9, SD 26·7). The age distribution of samples with available age information matched that of the population of Saudi Arabia (figure 1). Of the 8719 individuals with available demographic information, 4341 (50%) were male.

All serum samples from the general population were first screened for MERS-CoV-specific IgG by rELISA, yielding reactive results in 152 (1·52%, 95% CI 1·29–1·78) of 10 009 samples. Subsequent testing by rIFA and PRNT₅₀ confirmed less than 0·2% to be stage 1 and stage 2 seropositive (table 1; appendix). Because repeat samples were missing, the timepoint of seroconversion was established by IgM detection. Coronavirus IgM had been previously shown to be maintained for a maximum of 180 days after infection.²² Only one of 15 seropositive individuals had a reciprocal MERS-CoV IgM titre of at least 100, suggesting recent seroconversion (appendix). In accordance with our previous study,¹² seropositivity with rELISA was ten times higher than with rIFA. Seroprevalence did not differ between the two subcohorts

	Year of sampling	Total number	rELISA positive		Stage 1 seropositive		Stage 2 seropositive	
			n (%; 95% CI)	p value	n (%; 95% CI)	p value	n (%; 95% CI)	p value
General population	2012–13	10 009	152 (1·5%; 1·3–1·8)	NA	17 (0·2%; 0·1–0·3)	NA	15 (0·2%; 0·1–0·2)	NA
Camel shepherds	2014	87	6 (6·9%; 2·8–13·8)	p=0·0003	2 (2·3%; 0·3–7·4)	p=0·0009	2 (2·3%; 0·3–7·4)	p=0·0004
Slaughterhouse workers	2013	140	6 (4·3%; 1·8–8·7)	p=0·0224	5 (3·6%; 1·3–7·7)	p<0·0001	5 (3·6%; 1·3–7·7)	p<0·0001

Data are n (%; 95% CI) from a serosurvey, unless otherwise specified. p values refer to comparison with the general population cohort (χ^2 test with Yates correction two-tail test; OpenEpi). rELISA=recombinant ELISA. NA=not applicable.

Table 1: Middle East respiratory syndrome coronavirus antibodies in the general and subpopulations of Saudi Arabia

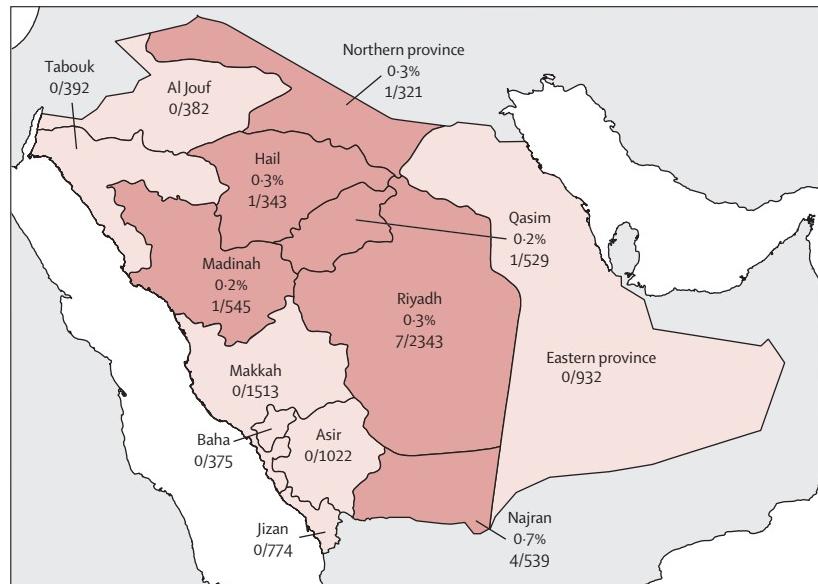


Figure 2: Serologically confirmed cases of Middle East respiratory syndrome coronavirus (MERS-CoV) in the general population of Saudi Arabia from Dec 1, 2012, to Dec 1, 2013

Red areas represent provinces that contain individuals with stage 2 seropositive MERS-CoV. Percentages show the number of stage 2 positive serum samples per number tested in each province (detailed numbers and full serological results per province in appendix).

(seven [0·16%] of 4244 patients in the primary health-care study group vs eight [0·14%] of 5765 in the burden of disease study cohort group, p=0·94; appendix). Men had a higher proportion of infections (11 [0·25%] of 4341) than did women (two [0·05%] of 4378; p=0·025). Cases were identified in four of six age groups without significant age preference (appendix).

Confirmed cases of MERS-CoV infection were identified in six of the 13 administrative provinces of Saudi Arabia, with higher numbers in central provinces that generally are more rural (figure 2). The 13 provinces were classified into seven central and six coastal provinces, on the basis of whether they had a shoreline. Significantly more confirmed infections were in central provinces (14 [0·26%] of 5479 samples) than in coastal provinces (one [0·02%] of 4529; p=0·003).

See Online for appendix

	Total	Mean age, years (SD)	Median age, years (IQR)	Reference	Date
Patients with serological evidence of past infection	18	43.50 (17.27)	42 (30.8–50.0)	This study	2012–13
Patients with clinically apparent infection*	482	53.76 (17.51)†	55 (41.0–68.0)	MERS-CoV spatial, temporal, and epidemiological information ²¹	2012–14
Index patients in household-contact study	24	54.29 (19.92)†	55 (46.3–69.3)	Drosten et al, 2014 ¹⁴	2013

Data from Saudi Arabia in 2012–14. MERS-CoV=Middle East respiratory syndrome coronavirus. *Notified primary or index cases. †Significantly different mean age compared with cases with serological evidence of infection (two-tailed Mann-Whitney U exact test); p=0.008 for clinically apparent cases and p=0.017 for index cases in household contact study.

Table 2: Age profiles in groups of patients with MERS-CoV infection in Saudi Arabia

Panel: Research in context

Systemic review

We searched PubMed with the terms “human coronavirus EMC” or “MERS-CoV” and identified 463 English-language reports published before Jan 20, 2015. Two previous studies^{14,15} reported an absence of Middle East respiratory syndrome coronavirus (MERS-CoV) antibodies in a few cohorts of serum samples obtained in Saudi Arabia from 2010 to 2012. One study investigated 266 individuals from the Eastern province.¹⁴ This study included 158 serum samples taken from children admitted to hospital for infections of the lower respiratory tract from May, 2010, to May, 2011, and 110 plasma samples from male blood donors taken during December, 2012. The other study¹⁵ assessed samples taken from 130 male blood donors from Jeddah during 2012 and 226 slaughterhouse workers in Jeddah and Makkah in October, 2012. No nationwide cross-sectional antibody surveys have been reported in any of the countries in the Middle East known to be affected by MERS-CoV, including Saudi Arabia, Jordan, United Arabic Emirates, Oman, Qatar, Kuwait, and Yemen.

Interpretation

We did the first nationwide cross-sectional serosurvey for MERS-CoV antibodies in the population of Saudi Arabia to explore the putative presence of unrecorded MERS-CoV infections. We noted that 0.15% of mainly male individuals in six of 13 had specific anti-MERS-CoV antibodies. Additionally, we identified a seroprevalence of MERS-CoV that was 15–23 times higher in camel-exposed individuals such as shepherds and slaughterhouse workers than in the general population. None of the individuals who tested positive for MERS-CoV antibodies had severe illness before or at the time of sampling. The mean age of all identified seroconverted individuals was significantly lower than that of patients with MERS admitted to hospital. In summary, our data suggest that middle-aged men with regular camel contact could sporadically introduce MERS-CoV into the general population. These subclinically infected individuals might represent the unknown source of infection for the many reported patients with MERS with no anamnestic camel exposure.

The proportion of both camel shepherds and slaughterhouse workers who were seropositive was significantly higher than in the general population (table 1). Seropositive individuals were identified in five of six age groups with even distribution (appendix).

Both registered patients (ie, patients with confirmed MERS)²¹ and index patients from the household study were significantly older than the serologically confirmed individuals in our present study (table 2; detailed laboratory results in the appendix).

Discussion

In our study, we identified antibodies against MERS-CoV in 0.15% of samples from the general Saudi Arabian population. The true number of infections is difficult to estimate because our sample consisted of only 10 009 (0.03%) of the whole population of 29 994 300 people. However, by combining two cross-sectional subcohorts we could cover all 13 provinces and achieved an age distribution of our sample that largely matched that of the general population (panel). All geographical regions of the country were sampled with a reasonably low variation in the proportions of population that were studied per province. To have optimum sample coverage per province, we included samples from an earlier study (unpublished report) for which detailed information about sex and age was not available for 26% of samples. Nevertheless, samples from both subcohorts should be similar in age and sex composition because both were previously obtained according to calculations by the National Census Bureau to represent the composition of the Saudi Arabian population. This similarity was lent support by the highly similar seroprevalence when we analysed both subcohorts separately. Even if we had only used data from the more thoroughly documented burden-of-disease study subcohort for which we had sex and age information for 97% of the samples, the overall seroprevalence would have been maintained (0.15% in the overall sample vs 0.14% in the subcohort).

To obtain an estimate of the total number of seropositive individuals in Saudi Arabia we did a straightforward extrapolation based on the proportion of stage-2-positive serum samples in both population subcohorts (excluding shepherd and slaughterhouse worker samples). Extrapolation by simple multiplication suggests that 44 951 seropositive individuals (95% CI 26 971–71 922) could exist in a total population of 29 994 300. Our serological assays might have missed some infections because the high specificity of rIFA and PRNT could encompass a reduction in sensitivity of the overall serological algorithm. However, even a projection based on rELISA results only would raise the estimated infection rate by about ten times, which is several orders

of magnitude lower than figures expected for a respiratory disease that continuously transmits between human beings. In accordance with our previous study,¹² seropositivity with rELISA was ten times higher than that with rIFA.¹² This rise can be attributed to the higher sensitivity of rELISA and by putative crossreactivity of low-affinity IgG, which is raised in individuals with recent infections from the common-cold coronavirus.²³

Despite low detection rates, antibodies were identified widely across Saudi Arabia, including in provinces where outbreaks in people have not been reported. Widespread acquisition of infections without subsequent causation of outbreaks corresponds to the concept of a predominantly zoonotic disease that does not readily spread between human beings. The raised prevalence in rural regions and the significantly increased proportion of infection in groups that are occupationally exposed to camels emphasises the importance of zoonotic acquisitions by dromedary camels.^{5–7} However, even though the analysed shepherd samples were collected in six different regions during the first months of 2014, the sampling was not randomised and samples were not strictly taken at the same time as those from the general population. Similar limitations apply for the slaughterhouse worker samples. Direct comparison of seroprevalences between these groups and the general population cohort might thus be biased. Matched case-control studies could be used to substantiate the raised seroprevalence in exposed groups.

Direct animal contact has been reported for only a few primary cases in Saudi Arabia and elsewhere. The younger age of serologically confirmed patients in our study compared with people with laboratory confirmed infection underlines the possibility that MERS-CoV might cause unrecognised infections that cause no or mild symptoms in healthy, younger (aged 15–44 years), and socially active populations. These subclinical infections could act as an unknown source for index patients who cannot recall contact with camels. Importantly, the predominance of men even within serologically confirmed cases emphasises a potential association with animal exposure because, typically, more men than women in Saudi Arabia associate with camels during leisure activities. Cases of MERS in younger people (aged <45 years) might seem mild or even subclinical, similar to cases in our study of household contact clusters,¹² in which contacts were younger than index patients and mostly asymptomatic. MERS might constitute a zoonotic disease that causes enough short-transmission chains in the young male population of Saudi Arabia, for which older and predisposed individuals constitute a sentinel population.

Contributors

MAM did the experiments, designed and coordinated the study, wrote the manuscript, analysed data, and generated figures. BM did the experiments and analysed data. VMC organised sample logistics, analysed data, and generated figures. MA-M and AT organised, coordinated, and supervised the samplings. DR and AS did the laboratory tests and analysed data. SA and PW analysed data. B-JB and EL provided diagnostic reagents and

analysed data. RFA, AMAs, AMAI, AMA-S, JAA-T, and AAAR designed and supervised the samplings. CD and ZAM designed and supervised the study, analysed and interpreted data, and wrote the manuscript.

Declaration of interests

We declare no competing interests.

Acknowledgments

We thank all staff of the Ministry of Health and the Ministry of Agriculture, Saudi Arabia; Sebastian Brünink and Stephan Kallies for technical assistance; and Anna Corman, Daniela Niemeyer, and the Institute for Medical Biometry, Informatics and Epidemiology of the University of Bonn Medical Centre for statistical advice. CD was supported by European Commission grant EMPERIE (contract number 223498) and ANTIGONE (contract number 278976), the German Centre for Infection Research, the Federal Ministry of Education and Research, and the German Research Council (grants 01KI1005 and DR 772/3-1, and MU 3564/1-1).

References

- European Centre for Disease Prevention and Control. Epidemiological update: Middle East respiratory syndrome coronavirus (MERS-CoV). March 8, 2015. <http://www.ecdc.europa.eu/en/publications/Publications/RRA-MERS-CoV-thirteenth-updatepdf> (accessed March 26, 2015).
- Assiri A, McGeer A, Perl TM, et al. Hospital outbreak of Middle East respiratory syndrome coronavirus. *N Engl J Med* 2013; **369**: 407–16.
- Cotten M, Watson SJ, Kellam P, et al. Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive genomic study. *Lancet* 2013; **382**: 1993–2002.
- Reusken CB, Haagmans BL, Müller MA, et al. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. *Lancet Infect Dis* 2013; **13**: 859–66.
- Alagaili AN, Briese T, Mishra N, et al. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. *MBio* 2014; **5**: e00884–14.
- Memish ZA, Cotten M, Meyer B, et al. Human infection with MERS coronavirus after exposure to infected camels, Saudi Arabia, 2013. *Emerg Infect Dis* 2014; **20**: 1012–15.
- Haagmans BL, Al Dhahiry SH, Reusken CB, et al. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. *Lancet Infect Dis* 2014; **14**: 140–45.
- Reusken CB, Farag EA, Jonges M, et al. Middle East respiratory syndrome coronavirus (MERS-CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar, April 2014. *Euro Surveill* 2014; **19**: 20829.
- Breban R, Riou J, Fontanet A. Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. *Lancet* 2013; **382**: 694–99.
- Cauchemez S, Fraser C, Van Kerkhove MD, et al. Middle East respiratory syndrome coronavirus: quantification of the extent of the epidemic, surveillance biases, and transmissibility. *Lancet Infect Dis* 2014; **14**: 50–56.
- Cauchemez S, Van Kerkhove MD, Riley S, Donnelly CA, Fraser C, Ferguson NM. Transmission scenarios for Middle East respiratory syndrome coronavirus (MERS-CoV) and how to tell them apart. *Euro Surveill* 2013; **18**: 20503.
- Drosten C, Meyer B, Müller MA, et al. Transmission of MERS-coronavirus in household contacts. *N Engl J Med* 2014; **371**: 828–35.
- Drosten C, Muth D, Corman V, et al. An observational, laboratory-based study of outbreaks of MERS-Coronavirus in Jeddah and Riyadh, Kingdom of Saudi Arabia, 2014. *Clin Infect Dis* 2015; **60**: 369–77.
- Gierer S, Hofmann-Winkler H, Albuiali WH, et al. Lack of MERS coronavirus neutralizing antibodies in humans, Eastern province, Saudi Arabia. *Emerg Infect Dis* 2013; **19**: 2034–36.
- Aburizaiza AS, Mattei FM, Azhar EI, et al. Investigation of anti-Middle East respiratory syndrome antibodies in blood donors and slaughterhouse workers in Jeddah and Makkah, Saudi Arabia, Fall 2012. *J Infect Dis* 2014; **209**: 243–46.
- Basulaiman M, El Bcheraoui C, Tuffaha M, et al. Hypercholesterolemia and its associated risk factors—Kingdom of Saudi Arabia, 2013. *Ann Epidemiol* 2014; **24**: 801–08.

- 17 Murray CJ, Ortblad KF, Guinovart C, et al. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; **384**: 1005–70.
- 18 Raj VS, Mou H, Smits SL, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 2013; **495**: 251–54.
- 19 Corman VM, Muller MA, Costabel U, et al. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. *Euro Surveill* 2012; **17**: 20334.
- 20 Buchholz U, Muller MA, Nitsche A, et al. Contact investigation of a case of human novel coronavirus infection treated in a German hospital, October–November 2012. *Euro Surveill* 2013; **18**: 20406.
- 21 Wikramaratna P, Rambaut A. MERS-CoV spatial, temporal and epidemiological information. June 18, 2013. <https://github.com/rambaut/MERS-Cases/blob/gh-pages/data/cases.csv> (accessed Oct 21, 2014).
- 22 Woo PC, Lau SK, Wong BH, et al. Longitudinal profile of immunoglobulin G (IgG), IgM, and IgA antibodies against the severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in patients with pneumonia due to the SARS coronavirus. *Clin Diagn Lab Immunol* 2004; **11**: 665–68.
- 23 Meyer B, Drosten C, Muller MA. Serological assays for emerging coronaviruses: challenges and pitfalls. *Virus Res* 2014; **194**: 175–83.